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Effects of temperature on larval American lobster (*Homarus americanus*): Is there a trade-off between growth rate and developmental stability?

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ABSTRACT

The American lobster supports the most economically valuable fishery in the Gulf of Maine and Atlantic Canada. Across much of its range, ocean temperatures have increased at rates faster than almost anywhere in the world. Studies of warming effects on larvae have largely focused on survival and development, but rarely have examined sub-lethal effects that could influence settlement and subsequent recruitment to the fishery. We explored how warming influences rate of development, survival, stress, and developmental stability of larval lobsters reared under four nominal temperatures: 14, 16, 18, and 22 °C. Our study is the first to evaluate the use of fluctuating asymmetry as a biomarker for developmental instability in larval American lobster. We also recorded total hemocyte counts in postlarvae as an indicator of stress, a novel technique for work in larval lobster. Development proceeded significantly faster as temperature increased, and cumulative survival was significantly positively correlated with temperature. However, postlarvae reared under temperature extremes exhibited elevated hemocyte counts and had significantly lower levels of variance in midline asymmetry compared to intermediate temperature groups. Together, this suggests that warmer temperatures may facilitate faster growth at the expense of increased physiological stress and a loss of genetic diversity, potentially affecting the species' ability to adapt to changing environmental conditions. Fluctuating asymmetry could prove to be a useful bioindicator for population resilience.

1. Introduction

Temperatures in the world's oceans are increasing rapidly (Reid and Beaugrand, 2012; Stocker et al., 2013), and some of the most accelerated rates of warming are occurring in the Northwest Atlantic (Sherman et al., 2009; Taboada and Anadón, 2012; Pershing et al., 2015). The Gulf of Maine region, for example, is warming at a rate of 0.4 °C per decade (Thomas et al., 2017), which is faster than 99% of the global oceans (Pershing et al., 2015). American lobsters (*Homarus americanus*) occupy this region and are a vital part of the marine ecosystem throughout the Northeastern United States and Canada (Holthuis, 1991). American lobsters have a preferred thermal niche of 16.5 ± 4 °C (Crossin et al., 1998), although waters spanning its geographic distribution vary along a 25 °C gradient, producing variation in growth rate, age/size at maturity, morphology, and body size across the species' range (Factor, 1995; Wahle et al., 2013).

The American lobster sustains the most economically valuable fishery in the Gulf of Maine and Atlantic Canada (Steneck et al., 2011), and this industry is valued in excess of two billion dollars (FAO, 2017). Lobster populations in Southern New England experienced a collapse more than ten years ago, which was coincident with elevated ocean temperatures and the emergence of epizootic lobster shell disease (Pearce and Balcom, 2005; Wahle et al., 2009). The severity of the recent rate of temperature increase in the lobsters' geographical region has raised concern regarding the consequences of elevated temperatures, particularly sub-lethal effects that could contribute to stress and disease susceptibly.

Previous research exploring the sub-lethal effects of temperature on American lobsters has focused primarily on benthic adults, and a number of these studies have explored changes in total hemocyte counts (THCs) and altered biochemical function as metrics of stress and immune health (Battison et al., 2003; Dove et al., 2005; Battison, 2006).

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Hemocytes are cells that are carried in the hemolymph and attack foreign invaders through phagocytosis and other mechanisms (Martin and Hose, 1995; Babcock et al., 2008). Studies on larval lobster stages have generally examined the direct effects of temperature on survival, rates of development, molting, and hatching success, but the effects of temperature on THCs have yet to be assessed in larvae (see Quinn, 2017 for recent review).

Although not yet explored in lobsters, fluctuating asymmetry (FA) is a metric that has been used in many taxa to assess developmental stability and is defined as random deviations of bilateral traits from perfect symmetry due to subtle variations in the developmental environment (Palmer and Strobeck, 2003). Both genetic and environmental stressors have been linked to elevated FA, including pesticides, parasitism, metals contamination, and suboptimal temperatures (see Polak, 2003; Beasley et al., 2013 for comprehensive reviews). Exposure to elevated temperatures has also been linked to increased levels of FA in developing larval fruit flies (*Drosophila melanogaster* and *D. buzzatii* – Imasheva et al., 1997), juvenile mussels (*Mytilus edulis* – Nishizaki et al., 2015), and adult isopods (*Asellus aquaticus* Linn. – Savage and Hogarth, 1999). Further, some studies have shown correlations between FA, parasitism, and disease susceptibility (Rantala et al., 2004; Møller, 2006; Thornhill and Gangestad, 2006; Morris et al., 2016). Although elevated temperatures may increase growth rate and survival in larval lobsters (e.g., Mackenzie, 1988; Barret et al., 2017), there may be consequences to rapid growth and FA could be a novel metric to highlight potential risks to this important species. This study seeks to examine the effects of temperature on survival, growth, hemocyte abundance, and FA to increase our understanding of the possible impacts of increasing temperatures on the health of larval lobsters.

2. Materials and methods

2.1. Study species

The American lobster inhabits hard-bottom substrate at depths commonly between 4 and 50 m along the Atlantic Coast of North America from Newfoundland, Canada, to North Carolina, USA (Herrick, 1911; Holthuis, 1991). Females can produce up to 10,000 eggs, which hatch in the shallows (< 20 m depth) during the summer months. Newly hatched lobsters proceed through three, pelagic larval stages (I-III) before molting into the final, postlarval (IV) stage. Unlike the three larval stages, postlarvae can swim with clear direction and speed (Cobb et al., 1989) and are negatively phototactic and spend less time at the surface as they swim down to the benthos (Herrick, 1911). As such, stage IV marks the point of transition, or settlement, from the pelagic to the benthic realm in the life history of the American lobster (Lavalli and Lawton, 1996).

2.2. Larval rearing

In the summers of 2016 and 2017, egg-bearing females were collected by the Maine Department of Marine Resources (ME DMR) Ventless Trap Survey and delivered to the University of Maine's Aquaculture Research Center (ARC) in Orono, ME. Females were housed individually in bins (36 cm wide × 54 cm long × 28 cm deep) within several recirculating seawater systems. Artificial seawater was mixed to a salinity of 34 ppt and a pH of 8.1 using Crystal Sea Marine Mix® and Kent Marine Superbuffer-dKH™, and temperature was maintained at 16 °C to provide the optimal egg-development environment (Factor, 1995). Females were observed every 10 h for newly-hatched larvae. Upon hatch, larvae were carefully removed from bins and counted. Larvae were then evenly distributed among four temperature treatments selected to span the steep latitudinal thermal gradient encountered across the species' range (Factor, 1995): 14, 16, 18, and 22 °C. Pilot efforts to rear larvae at colder temperatures (i.e., < 12 °C) produced too few postlarvae for analyses, thus precluding them from subsequent experiments.

We used four recirculating seawater systems (one for each temperature treatment) that consisted of a 227 L header tank, four 75 L replicate experimental tanks, a 114 L sump, and a 65 L biofilter. We held between 10 and 12 females at a time, and experimental tanks were stocked with larvae from at least three different females. Importantly, we did not continue to stock any individual tank for more than 48 h to reduce size differentiation among larvae, and larvae from each female were spread equally among the treatments to account for genetic differences. Depending on the number of larvae available during stocking, larval densities ranged from 3.5 to 14 larvae per L in experimental tanks. Larval tanks were highly aerated and lobsters were fed live *Artemia* spp. (Grade A Brine Shrimp Eggs, Brine Shrimp Direct, Inc., Ogden, Utah) twice a day at a density of 12 ml^{-1} to prevent cannibalism. Dissolved oxygen content, temperature, and salinity of the experimental tanks were monitored daily, and water quality was assessed weekly. The pH of each system was also assessed bi-weekly and maintained at 8.1 using Kent Marine Superbuffer-dKH™.

2.3. Biological endpoints

To assess development, a subset of 20 individual larvae was carefully netted and removed from each tank daily and staged using a Unitron® Z850 Zoom Stereo Microscope. Stage II larvae were distinguished from stage I larvae by the presence of pleopods on the abdomen; stage III larvae were distinguished by the enlargement of the claws and the presence of uropods on either side of the telson; and stage IV (postlarvae) were identified by well-defined and extended claws and a more typical lobster morphology (Herrick, 1895; Factor, 1995). Progression through these stages was determined as the date when at least 50% of the animals in a tank metamorphosed into the subsequent stage. Excluding stage IV, larvae were returned to experimental tanks following stage assessment. Cumulative survival was calculated as the proportion of stage I larvae initially stocked that successfully metamorphosed to stage IV.

2.4. Morphometric analyses

Upon reaching stage IV, postlarvae were removed from a given tank and assigned a unique identification number. Postlarvae were then placed in a clear Petri dish atop 6 mm × 6 mm grid paper and photographed using a Canon Rebel T5 camera. Photographs were taken on the "no flash" setting, and the lens was zoomed in so that lobsters could be seen in detail. Photographs from at least 20 postlarvae from each temperature treatment were blindly assessed using ImageJ2 software (Rueden et al., 2017). In triplicate, we measured the distance (in mm) from the middle of each eye to the midline of the body (Fig. 1). Average values were then used to calculate metrics of asymmetry.

2.5. Total hemocyte counts

Hemolymph was drawn from at least 12 postlarvae per temperature treatment to measure total hemocyte counts (THCs). Briefly, postlarvae were individually placed on a platform (a small crevice of a damp sponge) and viewed under a Unitron® Z850 Zoom Stereo Microscope. We used 6 mm BD® Insulin Syringes to draw as much of the animal's hemolymph as possible. Hemolymph was placed in pre-weighed glass vials containing 50 µl of fixative (10% buffered formalin in filtered, sterilized seawater) and the ratio of hemolymph to fixative was calculated using mass-differences. After aspirating the mixtures with a pipette, a 10 µl subsample was added to a hemocytometer (KOVA Glastic® Slide 10 with Grids). The total number of hemocytes was counted three times, and an average was calculated. This was repeated two more times, for a total of three subsamples per individual. Final counts were averaged and standardized to account for hemolymph: fixative dilutions among individuals (protocol modified from Dove et al., 2005).

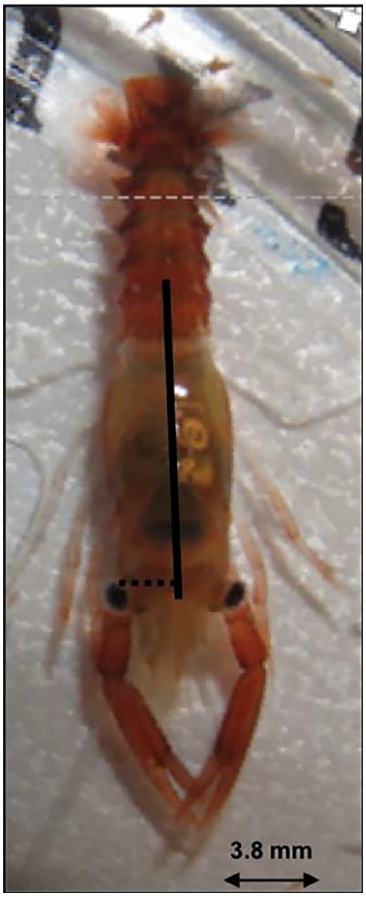

Fig. 1. Example image used for morphometric analyses of a stage IV larva. The solid line indicates the midline of the body and the dotted line depicts the measured distance from the center of one eye to this midline (this measurement is not pictured but was conducted for the other eye as well).

2.6. Statistical analyses

We used General Linear Models (GLMs) to determine the effects of temperature, year, and the interaction of year and temperature on the rate of development, cumulative survival, and THCs of postlarvae. We also explored whether stocking density had an effect on larval survival or rate of development using GLMs. We used Levene's Test to assess equal variance across groups and visually inspected data using histograms and q-q plots to assess normality. Time (in days) to stage II, stage III, and stage IV, as well as THC data, were log transformed, and cumulative survival data were square root transformed to meet test assumptions. Post hoc LSD tests were used to conduct pairwise comparisons across nominal temperature groups.

We calculated midline asymmetry (M_a) as the absolute difference between the distance from the middle of the right eye to the centerline of the body (R) and the middle of the left eye to the centerline of the body (L): $M_a = |R–L|$ (Palmer and Strobeck, 1986, 2003). We assessed temperature effects on midline asymmetry using a nonparametric Levene's Test because data did not meet test assumptions of a GLM. We determined that measurement error was insignificant, found no effect of trait size on fluctuating asymmetry, and conducted a series of preliminary tests to exclude other forms of asymmetry (i.e., directional asymmetry and antisymmetry) following the protocols outlined in Palmer and Strobeck (1986, 2003).

Finally, we performed bivariate correlation analyses between cumulative survival, time to stage IV, and nominal temperature treatment. While the post hoc LSD tests illuminated treatment-specific differences, this additional correlative approach allowed us to more fully understand the relationships among these specific experimental variables. All tests were performed using IBM® SPSS® Statistics Version 24.

Table 1
Results of GLMs for the effects of year and nominal temperature on time to stage II (A), time to stage III (B), and time to stage IV (C). *P* values indicating strong evidence for effects are shown in bold.

Source	df	*F*	*P*
(A)			
Corrected model	7	9.2	< **0.001**
Temperature	3	19.3	< **0.001**
Year	1	3.7	0.069
Temperature * Year	3	2.2	0.121
(B)			
Corrected model	7	42.4	< **0.001**
Temperature	3	97.9	< **0.001**
Year	1	0.12	0.738
Temperature * Year	3	5.5	**0.006**
(C)			
Corrected model	7	75.4	< **0.001**
Temperature	3	168.8	< **0.001**
Year	1	8.5	**0.009**
Temperature * Year	3	2.8	0.069

3. Results

Development time was significantly faster in higher temperature treatments, particularly for time to stage IV (Table 1, Fig. 2), which was significantly negatively correlated with nominal temperature ($R = -0.9$, $p < 0.01$). There was no significant year effect on time to stage II, but there was for time to stages III and IV (Table 1). However, this was driven solely by the significantly greater values observed in 2016 for the 14 °C treatment as determined by comparing means using a *t*-test (stage III: $t = 4.4$, $p < 0.01$; stage IV: $t = 2.7$, $p = 0.05$). Pairwise comparisons found no significant differences in the time to stage II between the 14 and 16 °C and between the 16 and 18 °C groups (LSD: $p > 0.1$); however, all other temperature treatments were significantly different across all developmental stages (LSD: $p < 0.05$; Fig. 2). We found no significant interactive effect between year and temperature treatment on the time to any stage (Table 1). There was also no significant effect of stocking density on rate of development for any stage (GLM, stage II: $F_{3,24} = 0.1$, $p = 0.9$; stage III: $F_{3,24} = 0.03$, $p = 0.9$; stage IV: $F_{3,24} = 0.05$, $p = 0.9$), indicating no effect of density on the overall trends.

Average cumulative survival was significantly affected by both year (GLM: $F_{1,20} = 4.8$, $p < 0.05$) and temperature (GLM: $F_{3,20} = 17.7$, $p < 0.001$) treatment, but there was no interactive effect (GLM: $F_{3,20} = 0.4$, $p = 0.73$; Fig. 3). In both years, mean cumulative survival was significantly positively correlated with nominal temperature ($R = 0.6$, $p < 0.01$). However, pairwise comparisons found no significant difference in cumulative survival between the 14 and 16 °C, 16 and 18 °C, 16 and 22 °C, and the 18 and 22 °C treatments in 2016 (LSD: $p > 0.05$). Similarly, we observed no significant difference in cumulative survival values between the 14 and 16 °C and the 18 and 22 °C treatments in 2017 (Fig. 3). Importantly, there was no significant difference in mean cumulative survival with each temperature treatment across years as observed using Student's *t*-tests (14 °C: $t = 0.13$, $p = 0.90$; 16 °C: $t = 1.71$, $p = 0.19$; 18 °C: $t = 1.09$, $p = 0.07$; 22 °C: $t = 1.41$, $p = 0.69$). There was also no significant effect of stocking density on cumulative survival (GLM: $F_{3,24} = 0.4$, $p = 0.8$), again suggesting variable stocking density did not impact our results.

THCs of postlarvae were significantly affected by temperature (GLM: $F_{3,\ 64} = 9.8$, $p < 0.001$; Fig. 4). Mean THCs for postlarvae reared at the extremes (14 and 22 °C) were not significantly different from one another but were significantly greater than mean THCs in postlarvae reared at both 16 and 18 °C (which were not significantly

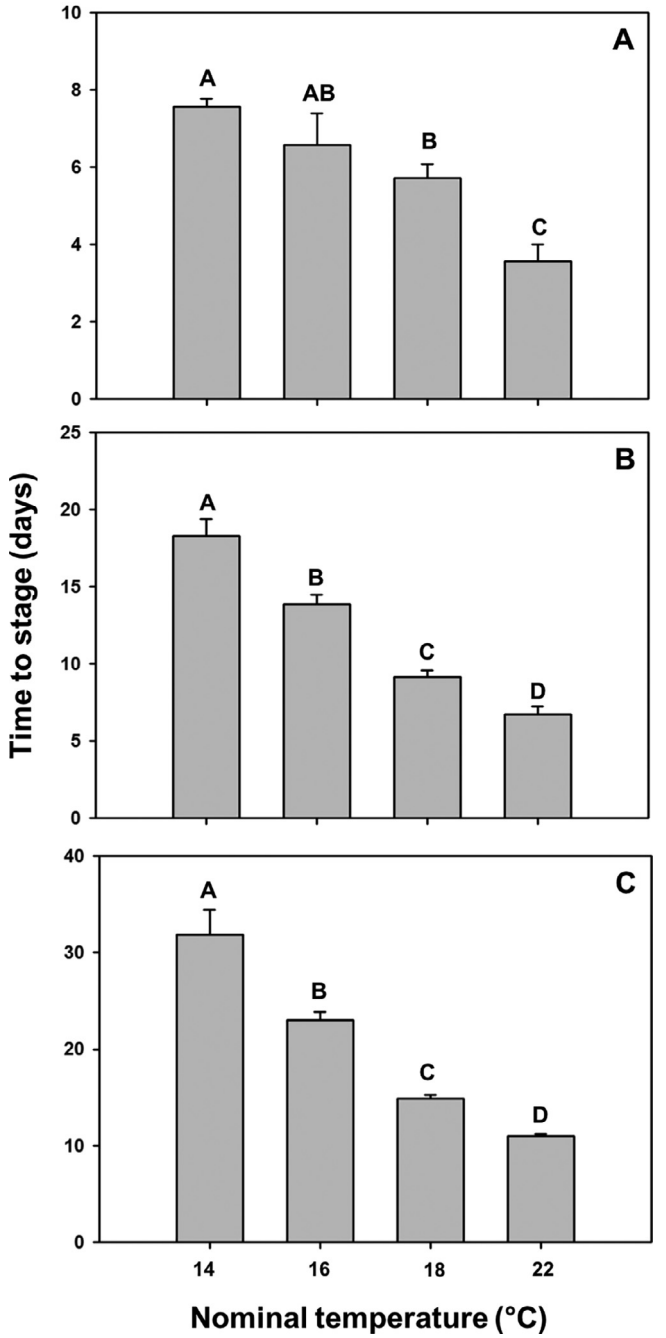

Fig. 2. Mean (+SE) time to stage II (A), stage III (B), and stage IV (C) for larvae reared under different nominal temperatures for 2016 and 2017 data combined. Letters indicate significant differences based on GLMs followed by post hoc LSD tests ($p < 0.01$).

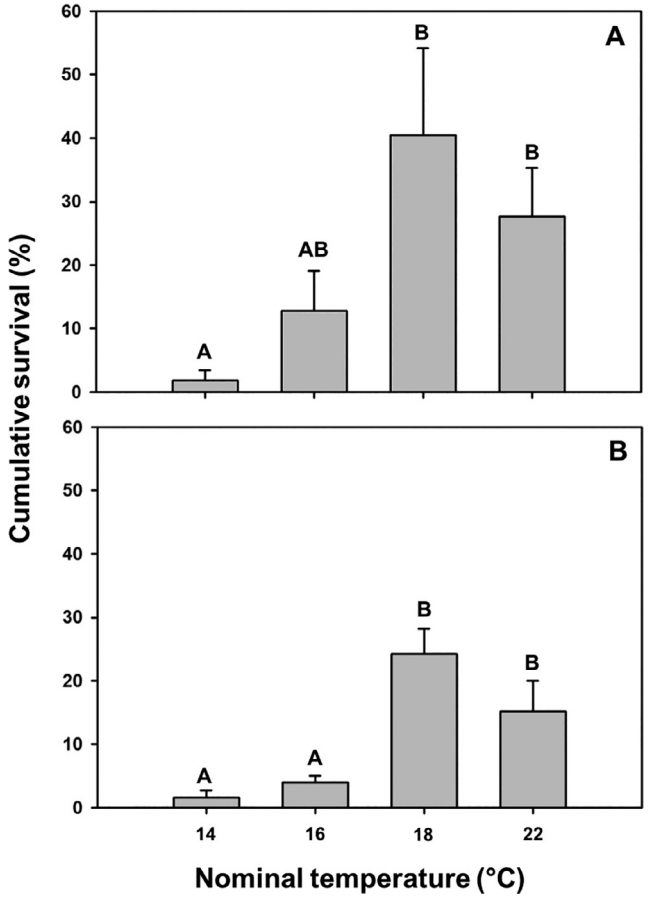

Fig. 3. Mean (+SE) cumulative survival for larvae reared under different nominal temperatures in 2016 (A) and 2017 (B). In 2016, mean (± SE) survival was 1.9 ± 1.5, 12.8 ± 6.2, 40.4 ± 13.7, and 27.6 ± 7.7% at 14, 16, 18, and 22 °C, respectively. In 2017, mean (± SE) survival was 1.5 ± 1.2, 4.0 ± 1.0, 24.2 ± 4.0, and 15.3 ± 4.8% at 14, 16, 18, and 22 °C, respectively. Letters indicate significant differences based on GLMs followed by post hoc LSD tests ($p < 0.01$).

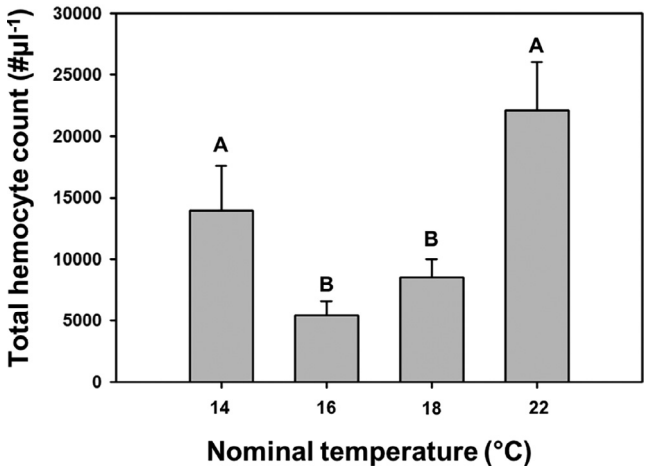

Fig. 4. Mean (+SE) total hemocyte count (THC) for larvae reared under different nominal temperatures in 2017. Letters indicate significant differences based on GLMs followed by post hoc LSD tests ($p < 0.01$).

different from one another; Fig. 4).

We found a significant effect of temperature on the variance in midline asymmetry (Nonparametric Levene's Test: $F_{3,222} = 4.8$, $p < 0.01$; Fig. 5). Variance in midline asymmetry was higher for postlarvae reared at 16 and 18 °C compared to postlarvae reared at 14 and 22 °C, with pairwise comparisons demonstrating significant differences between postlarvae reared at 14 and 16 °C, 14 and 18 °C, and between those reared at 16 and 22 °C (LSD: $p < 0.05$; Fig. 5).

4. Discussion

Our work is the first to evaluate the use of FA as a biomarker for developmental instability in response to stress in larval *H. americanus*. We expected postlarvae reared under temperature extremes to exhibit greater levels of midline FA, but we found that postlarvae reared at 14 and 22 °C had significantly lower levels of variance in midline FA

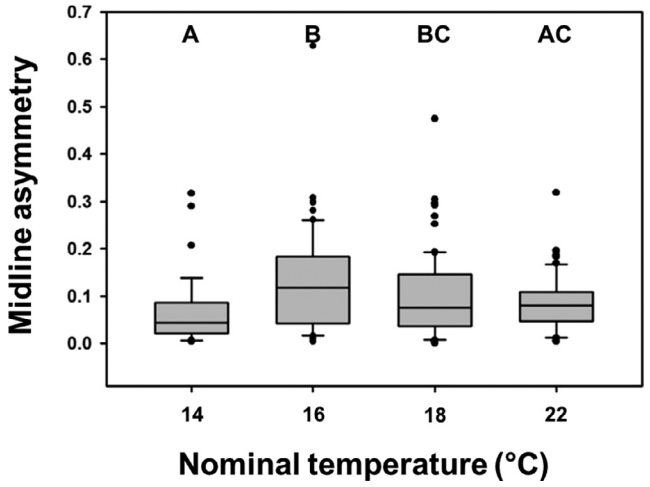

Fig. 5. Boxplots depicting the midline asymmetry for 2016 and 2017 data combined. Letters indicate significant differences in variance via a nonparametric Levene's Test followed by post hoc LSD tests ($p < 0.01$). The upper and lower quartiles are represented as the top and bottom ends of each box, respectively, and the median is marked by the horizontal line within each box. Lines extending vertically above and below the box indicate the variability outside of the upper and lower quartiles, and outliers are represented by individual points.

compared to the intermediate temperature groups (Fig. 5). There has been much debate about the utility of FA as an indicator of developmental instability (Palmer and Strobeck, 1986; Leung and Forbes, 1996). Although recent meta-analyses suggest that it is a reliable biomarker (Beasley et al., 2013), expression of FA in response to environmental stress is both species- and trait-specific (Lazić et al., 2013; Klisarić et al., 2014). Elevated FA has been linked to elevated levels of pollution, including exposure to heavy metals in brown trout (*Salmo trutta fario* – Monna et al., 2011), tributyltin in surf crabs (*Ovalipes trimaculatus* – Lezcano et al., 2015), Roundup Original® in tadpoles (*Physalaemus cuvieri* – Costa and Nomura, 2016), and urbanization in lizards (*Podarcis muralis* – Lazić et al., 2013). Exposure to sub-optimal temperatures also produced increased levels of FA in developing larval fruit flies (*Drosophila melanogaster* and *D. buzzatii* – Imasheva et al., 1997), juvenile mussels (*Mytilus edulis* – Nishizaki et al., 2015), and adult isopods (*Asellus aquaticus* Linn. – Savage and Hogarth, 1999). This previous research has demonstrated a link between increased variance in traits of populations exposed to novel environments or pollutants, suggesting that elevated variance arises as populations struggle to adapt to a changing environment (see references within Orlando and Guillette, 2001); however, research also suggests that human-induced stress produces a loss of genetic diversity in populations via genetic erosion (van Straalen and Timmermans, 2002). Genetic erosion can be caused by genetic bottlenecks, mutations, altered migration patterns, and directional selection of tolerant genotypes in response to stress, and it can lead to the loss of alleles, reduced population growth, and increased susceptibility to further genetic erosion (van Straalen and Timmermans, 2002; Ribeiro and Lopes, 2013). For example, scientists observed reduced genetic diversity in populations exposed to trace metal pollution (e.g., amphibians – Fasola et al., 2015; cladocerans *Daphnia longispina* – Venâncio et al., 2016; tiger prawn *Penaeus monodon* – Rumisha et al., 2017), climate-induced habitat loss and fragmentation (e.g., chipmunk *Tamias alpinus* – Rubidge et al., 2012; tree frogs *Litoria ewingii* and *L. raniformis* – Potvin et al., 2017), and human-induced habitat destruction (e.g., snails *Littoraria subvitatta* – Nehemia et al., 2017). At first glance, our results might appear to indicate lower levels of developmental instability of postlarvae reared under extreme temperatures compared to the intermediate temperature treatments. However, we suggest that only those individuals with genotypes tolerant of the extreme temperature treatments were able to survive this thermal stress, potentially altering morphological development that manifested as reduced variability in midline FA. Moreover, this observed decline in variation of FA suggests that postlarval populations reared under our warmest temperature treatment may have lower adaptive capacities to deal with additional stressors (van Straalen and Timmermans, 2002). This is particularly important in the context of climate change, in which postlarvae will have to deal with numerous other stressors in addition to warming temperatures. Individuals (and thus populations) that are tolerant of one stressor may be sensitive to a second stressor, potentially enhancing genetic erosion in a population via a second round of gene loss (van Straalen and Timmermans, 2002; Venâncio et al., 2016). Future efforts should continue to explore the utility of FA as an indicator of developmental instability in larval lobsters, particularly in response to multiple stressors.

We assessed physiological stress levels by quantifying total hemocyte counts (THCs), a common measure of stress that has not been previously implemented in work on larval lobsters. Invertebrates lack an adaptive immune system, and as such hemocytes play a critical role in the innate immune response (Cerenius and Soderhall, 2011; Adamo, 2012). Free hemocytes rapidly circulate within the hemolymph, surveying for areas of damage and identifying self from non-self (Babcock et al., 2008). In *H. americanus*, hemocytes assist in wound healing via clotting, harden the exoskeleton, and remove foreign material and pathogens via phagocytosis (Martin and Hose, 1995; Battison et al., 2003; Mori and Stewart, 2006). Most previous work on hemocytes in invertebrates has focused on understanding changes in THCs in the context of pathogen exposure (e.g., white shrimp *Litopenaeus vannamei* – Cheng et al., 2006; giant prawn *Macrobranchium rosenbergii* – Chang et al., 2011; mud crab *Scylla paramamosain* – Zhou et al., 2018). However, as the innate immune response and the stress response are intimately tied in invertebrates (Adamo, 2012), THCs have also been measured in the context of other stressors. For example, researchers linked elevated THCs to acute stress events in crickets (*Gryllus texensis* – Adamo, 2010) and western rock lobster (*Panulirus cygnus* – Jussila et al., 2001). Additionally, researchers observed a decline in THCs in white shrimp (*Litopenaeus vannamei*) in response to cold shock, suggesting a depressed immune response relative to preferred thermal conditions (Chang et al., 2009).

Previous work on adult *H. americanus* found an increase in THCs in response to elevated temperatures (Dove et al., 2005). Moreover, this increase was accompanied by a 60% reduction of phagocytotic activity of hemocytes within just two weeks of exposure to stressfully warm conditions, as well as severe pH acidosis, suggesting deleterious effects of warming temperatures on lobster physiology (Dove et al., 2005). Similarly, we observed elevated THCs in postlarvae reared under 22 °C compared to those reared under 16 and 18 °C, suggesting an elevated stress response in these treatments. One might expect physiological responses to increase in lobsters as their external environmental temperature increases because they are ectotherms; however, the fact that we did not observe a linear increase in THCs with temperature treatment points to a more regulated stress response independent of temperature. We also found an increase in THCs in postlarvae reared at 14 °C that was not statistically different from that of the 22 °C group, suggesting that these larvae also experienced stressful thermal conditions during development. A similar pattern was found in the freshwater amphipod (*Gammarus pulex*) when exposed to a range of environmentally-relevant temperatures (9–17 °C), whereby THCs were reduced in individuals reared at intermediate temperatures compared to both the warm and cool extreme temperatures tested (Labaude et al., 2017). Amphipods reared at the extremes also exhibited a reduction in their ability to clear an *Escherichia coli* infection, suggesting a potential breakdown in the immune response as a consequence of temperature-induced stress (Labaude et al., 2017). Since some crustaceans exhibit greater levels of tissue damage at extreme temperatures (Madeira et al., 2015), it is possible that the elevated THCs observed in our study are

related to wound healing and the breakdown of damaged cells (as suggested for *G. pulex* – Labaude et al., 2017). Continued warming conditions could increase disease susceptibility in recently settled American lobster if they are allocating more energy toward fixing damaged cells, potentially increasing post-settlement mortality. However, it is important to note that THCs are just one metric of stress, and future efforts should explore other indicators (e.g., levels of glucose, crustacean hyperglycemic hormone, or *L*-lactate – Basti et al., 2010). Additionally, future efforts should focus on not only measuring other parameters of hemolymph chemistry (e.g., phagocytotic activity and pH), but also understanding the potential interactions between temperature stress and immune function in postlarvae.

Development rate was significantly enhanced under warming conditions, which aligns with previous work on larval *H. americanus* (Hadley, 1906; Templeman, 1936; MacKenzie, 1988; Waller et al., 2017; Barret et al., 2017). Mean cumulative survival was also significantly elevated by rearing temperature, which increased up to 18 °C (24–40%) with an insignificant reduction in survival at 22 °C (15–27%) (Fig. 3). This finding is consistent with previous studies showing increased survival in larval *H. americanus* at elevated temperatures (MacKenzie, 1988; Barret et al., 2017). Although Waller et al. (2017) described significant reductions in survival of larvae reared at elevated temperatures, that study reports considerably lower cumulative survival (< 2.0%). This may have been due to cannibalism (Waller et al., 2017), which could be expected to increase at higher temperatures to compensate for increased feeding and growth rates. In our study, vigorous aeration was necessary to reduce agonistic interactions and limit the incidence of cannibalism. However, Quinn et al. (2013) also found low cumulative survival (< 4.0%) in larvae reared individually at 20 °C, eliminating the possibility of cannibalism-induced mortality. Rearing larval lobsters can be technically challenging, and numerous factors including system design, diet, and water quality can play a substantial role in survival outcomes. Notably, survival was lowest in our 14 °C treatment, and pilot efforts to rear larvae at colder temperatures (10–12 °C) produced too few for meaningful analyses. This is consistent with Barret et al. (2017) and Mackenzie (1988) which generally found < 5.0% survival at 10 °C. Variation in larval development is dependent upon both larval origin (i.e., cold- vs. warm-water maternal origin – Quinn et al., 2013), and the thermal variability encountered in the natural environment (Quinn and Rochette, 2015). Females used in this study were collected from southern Maine waters, so it is possible that their larvae perform better under warmer conditions compared to those from females of waters near colder portions of the species' range. More work is needed to understand the geographical variation in responses to warming temperatures, particularly in the context of a changing climate.

Overall, this work adds to our understanding of the indirect effects of rising temperatures on larval *H. americanus*, providing insight into the potential sub-lethal effects of ocean warming that could assist modeling efforts to link larval settlement to subsequent recruitment into the fishery (e.g., Barret et al., 2017; Quinn et al., 2017). Our findings suggest that larvae reared under warming conditions exhibit enhanced rates of development and higher rates of survival, but these benefits are potentially outweighed by the increase in stress level as indicated by elevated THCs. We also suggest that reduced variation in midline FA may indicate a reduced capacity to deal with additional stressors, although more work is needed to understand long-term consequences. Taken together, these data indicate a potential tradeoff between enhanced growth and sub-lethal impacts on larval physiology under warming conditions. This is the first study to explore the utility of FA as an indicator of developmental instability in *H. americanus*, and future efforts should explore additional morphometric traits, as well as the potential for geographic variation in these metrics. Future research should also focus on understanding more downstream effects of both FA and THCs in the context of post-settlement survival, particularly as these parameters relate to interspecific interactions and foraging behaviors. Finally, it is important to acknowledge that ocean warming is not occurring in isolation, and future efforts should examine the potential interactive effects of temperature, ocean acidification, and salinity on *H. americanus* larval survival, development, and fitness post-settlement.

5. Conflicts of interest

We declare that we have no competing interests.

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